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Dose-Response Study of Chloroform Carcinogenesis in the Mouse and Rat Status Report

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16. ABSTRACT
Chloroform is being administered to male Osborne-Mendel rats and to femal B6C3F1 mice at concentrations of 0 (negative control), 200, 400, 900 or 1800 ppm in the drinking water. Matched control groups of both species receive a volume of water identical to that consumed by the corresponding 1800ppm groups. At this writing, the animals have completed 23 months on test. Negative control and $CHCl_3$-treated rat groups have shown typical growth curves, with dose-related relative decrements in body weight evident throughout the study. Following decreases in $CHCl_3$ groups during the first 8 weeks, rat water consumption values have continued to increase slowly, but persistent relative dose-related decrements are evident. No initial treatment-related decrements are evident. No initial treatment-related mortality was seen in the rats. Survival is 21,41, 45, 76, 70 and 64% for the negative control, 200, 400, 900, 1800 ppm and matched control groups, respectively. Survival values for mice at three weeks were 99, 94, 74 and 76% for the 200, 400, 900 and 1800 ppm groups, respectively. Mortality was apparently related to markedly decreased fluid consumption among some of the treated mice. Subsequent mortality has been less than 15% for all mouse groups. Except for acclimation effects furing the first 2-3 weeks, body weights for the treated mouse groups have been generally within 10% of negative control values. Tissue changes in decedents have been similar in treated and control groups, both in rats and mice. Terminal sacrifice and histologic evaluations will be initiated after completion of 24 months on test.

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Dose-Response Study of Chloroform Carcinogenesis in the Mouse and Rat: Status Report

by Theodore A. Jorgenson,* Carol J. Rushbrook* and David C. L. Jones*

Chloroform is being administered to male Osborne-Mendel rats and to female B6C3F1 mice at concentrations of 0 (negative control), 200, 400, 900 or 1800 ppm in the drinking water. Matched control groups of both species receive a volume of water identical to that consumed by the corresponding 1800 ppm groups. At this writing, the animals have completed 23 months on test. Negative control and $CHCl_3$-treated rat groups have shown typical growth curves, with dose-related relative decrements in body weight evident throughout the study. Following decreases in $CHCl_3$ groups during the first 8 weeks, rat water consumption values have continued to increase slowly, but persistent relative dose-related decrements are evident. No initial treatment-related decrements are evident. No initial treatment-related mortality was seen in the rats. Survival is 21, 41, 45, 76, 70 and 64% for the negative control, 200, 400, 900, 1800 ppm and matched control groups, respectively. Survival values for mice at three weeks were 99, 94, 74 and 76% for the 200, 400, 900 and 1800 ppm groups, respectively. Mortality was apparently related to markedly decreased fluid consumption among some of the treated mice. Subsequent mortality has been less than 15% for all mouse groups. Except for acclimation effects during the first 2-3 weeks, body weights for the treated mouse groups have been generally within 10% of negative control values. Tissue changes in decedents have been similar in treated and control groups, both in rats and mice. Terminal sacrifice and histologic evaluations will be initiated after completion of 24 months on test.

Introduction

There have been several studies of tumorigenesis induced in animals by chloroform. Eschenbrenner (*1*) reported hepatomas induced in female Strain A mice receiving 0.3 or 0.6 mg/kg of chloroform by gavage every four days for three months. In a study conducted by Hazleton Laboratories in 1976 for the National Cancer Institute (*2*), chloroform was administered by gavage five times per week for 78 weeks to Osborne-Mendel rats at 90–250 mg/kg/day and to B6C3F1 mice at 100–500 mg/kg/day. Kidney epithelial tumors were induced in male rats and hepatocellular carcinomas in mice of both sexes. Decreased survival rates and body weights were reported in treated rats but were not observed in the mice. Theiss et al. (*3*) reported no increase in lung adenomas in Strain A mice receiving intraperitoneal injections of chloroform at 80 or 200 mg/kg three times per week for 8 weeks, or two injections at 400 mg/kg.

The present study was designed to evaluate the chronic toxicity and tumorigenicity of chloroform administered in the drinking water to male Osborne-Mendel rats and to female B6C3F1 mice over a wide range of dose levels.

Methods

Animals

The species and strains of rodents used were those in the earlier study conducted by Hazleton. Rats were received from CAMM Research, and mice from Charles River. Mice were housed five

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per cage in 48.3 × 26.7 × 15.2 cm (19 × 10.5 × 6 in.) polycarbonate cages containing hardwood chip bedding (AbSorbDri). Cages were changed once a week at the same time as the body weights and water consumption were recorded.

Rats were housed two per cage in 43.2 × 21.6 × 20.3 cm (17 × 8.5 × 8 in.) polycarbonate cages containing the same hardwood chip bedding. Cages were changed twice a week: once when body weights and water consumption were recorded and again when water consumption was recorded for the second time in the week and the cages and racks were rotated.

Animals were assigned to experimental groups and cages using a table of random numbers.

For both species cages were rotated on the racks once a week. The racks were rotated on the same schedule within the room to ensure similar conditions for all animals throughout the study.

Control animals were housed in rooms as similar as possible but separate from the chloroform-treated groups.

Chemical Supply

The chloroform used in this program was pesticide quality purchased from Matheson Coleman Bell. The chloroform was analyzed for purity, concentration and stability in the drinking water. Before use, all chloroform was distilled to remove contaminants (diethyl carbonate, ethanol, and phosgene). Distilled water was used throughout the study for control drinking water as well as for the chloroform solutions.

Analytical Chemistry

Quantitative analyses for chloroform in air, water, feed and blood; distillation of chloroform and determination of impurity levels; determination of liver fat/organ weight ratios; and routine monitoring of feed lots for PCBs and chlorinated pesticides were performed. Ambient air in each experimental room was analyzed once every 6 months for chloroform concentrations. The Purina Laboratory Chow was analyzed annually for chloroform concentration with each shipment being analyzed for PCBs and chlorinated pesticides. Chloroform in blood from additional rats placed on test for this purpose was analyzed at 3, 6, 12, and 18 months and will be evaluated at 24 months on study. Once each month the stock solutions prepared on Tuesday were analyzed for chloroform. Additionally, samples from water bottles were analyzed once each month on the Friday following the stock solution analysis. Liver fat/liver weight ratios were examined at 3 and 6 months in both rats and mice.

Dose Levels and Number of Animals

The negative control (0 ppm) and 200 ppm rat groups were each composed of 330 animals. There were 150 rats in the 400 ppm group and 50 in the 900 ppm, 1800 ppm and matched control (M) groups. The negative control and 200 ppm mouse groups each had 430 animals, while the numbers of mice in the other groups were identical to the corresponding rat groups.

In addition to the above numbers of animals, there were 80 additional male rats per group for blood work and 10 additional male rats in all but the matched control group for liver fat analyses at 90 days on test. With the exception of the matched control group, each of the mouse groups had 20 additional mice. Ten mice per group were removed at 3 months and at 6 months for liver fat analyses.

Observations

Food intake and animal health and activity were noted daily, 7 days a week. Body weights were recorded weekly until stable (18 weeks for the rats and 14 weeks for the mice). Thereafter, body weight measurements were recorded monthly. Water consumption was measured twice weekly by weighing the water bottles during the two changes in water each week.

Hematology and Blood Chemistry

Blood parameters listed in Table 1 were evaluated on 20 male rats from each experimental group at 6, 12, and 18 months, and will be evaluated at 24 months on test.

A Coulter Counter Model S was used to determine red and white cell counts, hemoglobin, hematocrit, MCV, MCH, and MCHC. Differential counts were conducted manually. The chemical analyses were run on a Sequential Multiple Analysis Plus Computer (SMAC) system.

These additional animals were handled in the same manner as those in the lifetime study. At the time of sacrifice for blood analyses, we performed a gross necropsy and collected and saved the appropriate tissues in 10% neutral buffered formalin.

Gross and Microscopic Pathology

When any animal in these lifetime studies appeared unlikely to survive until the next scheduled observation, it was sacrificed. This aggressive sacrifice was intended to prevent the loss of information that could occur through autolysis or cannibalization.

All decedents were given a complete gross nec-

Table 1. Blood parameters evaluated.

Hematology	Clinical chemistry	
Hematocrit	Triglycerides	Glucose
Hemoglobin	Total bilirubin	BUN
Red cell count	SGOT	Creatinine
White cell count	SGPT	Uric acid
Differential count	LDH	Na^+
MCV	Alkaline phosphatase	K^+
MCH	Total iron	CO_2
	Total protein	Cl^-
MCHC	Albumin	Calcium
	Globulin	Phosphorus
	A/G ratio	Balance (Na-(Cl + CO_2))
		Cholesterol

Table 2. Measured chloroform concentrations in water bottles in percent of nominal values.[a]

Month	Percentage of nominal values at various chloroform levels			
	200 ppm	400 ppm	900 ppm	1800 ppm
1	93	94	88	76
2	84	93	95	94
3	104	108	80	94
4	92	84	89	89
5	99	102	100	99
6	106	94	90	73
7	106	100	101	98
8	107	97	96	98
9	103	104	105	103
10	104	104	100	94
11	95	96	91	88
12	89	88	88	83
13	96	98	94	97
14	90	95	92	89
15	100	104	100	97
16	108	100	100	105
17	100	103	121	96
18	91	87	95	94
19	104	102	101	98
20	104	104	101	105
21	104	100	107	96
22	92	93	90	79
23	105	100	110	109
24	117	111	109	99

[a]Mean of ten samples per level per month.

ropsy as defined in the Guidelines for Carcinogen Bioassay in Small Rodents, DHEW Publication No. (NIH) 78-23, Revised 1978, including examination of external surfaces and body orifices, and examination and fixation of all of the following: gross lesions, tissue masses or suspect tumors and regional lymph nodes, skin, mandibular lymph node, mammary gland, salivary gland, larynx, trachea, cecum, colon, rectum, mesenteric lymph node, liver, thigh muscle, lungs and bronchi, heart, thyroids, parathyroids, esophagus, stomach, duodenum, jejunum, ileum, spleen, kidneys, adrenals, bladder, seminal vesicles, prostate, sciatic nerve, sternebrae or vertebrae, or femur (plus marrow), costochondral junction, ribs, thymus, gall bladder, pancreas, testes, ovaries, uterus, nasal cavity, brain, pituitary, eyes and spinal cord. All tissues and organs were fixed in 10% neutral buffered formalin.

The following tissues were processed and read histologically, with all others being stored in fixative: suspect tumors and gross lesions, liver, kidney, regional lymph nodes, urinary bladder, esophagus, adrenals, spleen, stomach, small intestine, colon and lung. All rat slides were read by one pathologist, and all mouse slides were read by another pathologist.

Results

Since this study is still in progress, the results presented here are necessarily incomplete. Nonetheless, they provide a basis for evaluating the progress of the study and some information about the chronic effects of chloroform.

Table 2 shows the results of the analyses of the chloroform solutions in the drinking bottles. Chloroform concentrations varied somewhat over the course of the study, but were generally within ten percent of the nominal values. Clearly, there were no overlapping values between adjacent dose groups.

Survival, water consumption, and body weight patterns for the rats are shown in Figure 1. Survival has been essentially directly proportional to the chloroform dose level; lowest for the negative controls, next lowest for the 200 and 400 ppm groups, and highest for the matched controls and the 900 and 1800 ppm groups. Water consumption has increased slowly over the course of the study in all groups, with the amount consumed inversely proportional to dose level during the first 18 months, then a clustering of the negative control, 200 and 400 ppm groups at a relatively higher level, the 900 ppm group in the middle, and the 1800 ppm group at a lower level. Body weight patterns have shown typical growth curves, the values at any time point being inversely proportional to dose level. Values for the matched control group were above those for the 1800 ppm group during the first year, but the latter group has now caught up with its matched controls.

Survival, water consumption and body weight patterns for the mice are illustrated in Figure 2. Unlike the rats, there was significant early mortality in the treated mice, particularly at 900 and 1800 ppm. From the second through the eighteenth month very few animals died, but by the twenty-third month some mortality, proportional to dose level,

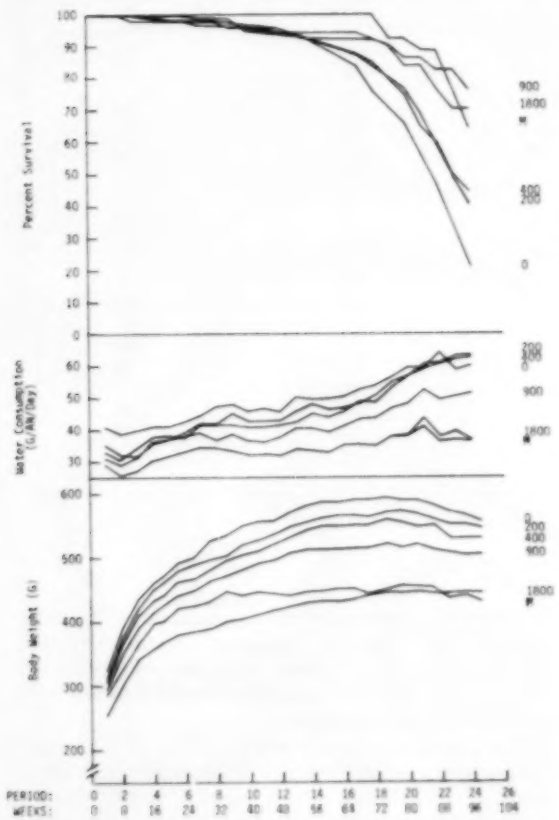

FIGURE 1. Survival, water consumption and body weight in male Osborne-Mendel rats receiving chloroform in the drinking water. Doses are indicated in ppm; M indicates matched control group receiving an amount of water equal to that consumed by the 1800 ppm group.

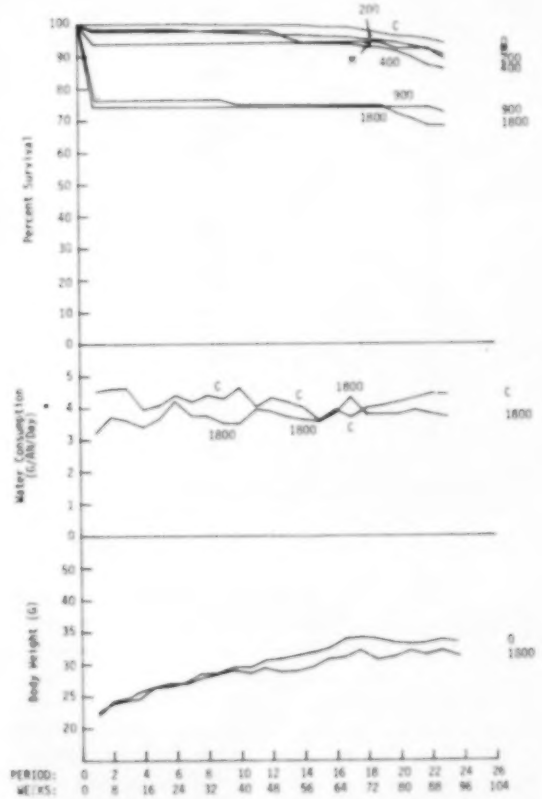

FIGURE 2. Survival, water consumption and body weight in female B6C3F1 mice receiving chloroform in the drinking water. Doses are indicated in ppm; M indicates matched control group receiving an amount of water equal to that consumed by the 1800 ppm group.

Table 3. Mean percent liver fat in animals receiving chloroform in the drinking water.

		3 Months		6 Months	
	Dose group	*N*	%	*N*	%
Rats	0 ppm	10	3.3	20	4.5
	M	—	—	18	4.6
	200 ppm	10	3.3	20	4.5
	400 ppm	10	3.2	20	4.6
	900 ppm	10	3.6	19	4.8
	1800 ppm	10	3.5	19	5.1[a]
Mice	0 ppm	10	3.3	7	5.8
	200 ppm	10	3.5	10	7.9[a]
	400 ppm	10	3.9[b]	10	6.8
	900 ppm	10	4.5[b]	6	7.1[a]
	1800 ppm	8	6.4[b]	8	10.4[b]

[a] $p \leq 0.05$.
[b] $p \leq 0.01$.

was apparent. Both water consumption and body weights were narrowly distributed among the various groups. The values for the two extreme groups, the negative controls and the 1800 ppm group, are shown for illustration. Water consumption for the mice was essentially stable throughout the study. Body weight increased more or less linearly, then reached a plateau beginning at about 18 months.

Although mean blood levels of chloroform were assessed in the rats at 3, 6, 12 and 18 months, there was difficulty in obtaining reliable data during the first year. For the 18-month assay, whole blood was used instead of serum, and reliable results were obtained. Values were 0.7, 0.8, 7.5, 22.1, 75.4 and 124 ppb for the negative controls, matched controls, and the 200, 400, 900 and 1800 ppm groups, respectively.

The percent fat in the liver of the rats and the mice at 3 and at 6 months is shown in Table 3. For the rats, there was no apparent increase in liver fat content in the treated groups at 3 months, but at 6 months there was a significant increase in the 1800 ppm group. In the mice, significant increases in liver fat were apparent at 400-1800 ppm at 3 months and at 900 and 1800 ppm at 6 months.

The hematologic findings in the rats at 6, 12 and 18 months are shown in Tables 4–6. WBC values were lower in the 1800 ppm group and in the matched controls at 6 and at 12 months. The differences in erythrocyte and hemoglobin parameters at 12 months are consistent with hemoconcentration in the treated groups, but no significant differences were apparent at 18 months.

The blood chemistry data for male rats sacrificed at 6, 12 and 18 months are shown in Tables 7–9. Although various parameters differed significantly from the negative control values at some time points, there were a number of apparent trends. Chloride,

Table 4. Summary of hematology data for male Osborne-Mendel rats receiving chloroform in their drinking water: 6-month sacrifice.

			Chloroform			
Parameter	Control	Matched control	200 ppm	400 ppm	900 ppm	1800 ppm
WBC × 10^3/mm^3	6.8	4.8[a]	6.6	6.5	6.8	5.5[a]
RBC × 10^6/mm^3	8.06	8.14	8.19	8.07	8.04	7.98
Hgb, g-%	15.3	15.4	15.5	15.3	15.2	15.3
Hct, %	44.2	44.3	44.4	43.6	43.7	44.0
MCV, μ^3	55	55	54	54	55	55.
MCH, $\mu\mu$g	18.9	18.7	18.8	18.7	18.8	19.0
MCHC, %	34.7	34.7	35.0	35.0	34.9	34.8
Differential						
PMN,%	10.7	14	13	12	11	12
Bands, %	0	0.1	0.1	0	0.1	0
Lymphocytes, %	85	82	84	85	86	84
Monocytes, %	2.5	2	2	2	2	2
Eosinophils, %	2	2	1.6	1	1	2
Basophils, %	0	0	0	0	0	0

[a] $p \leq 0.05$.
[b] $p \leq 0.01$.

Table 5. Summary of hematology data for male Osborne-Mendel rats receiving chloroform in their drinking water: one-year sacrifice.

Parameter	Control	Matched control[a]	Chloroform 200 ppm	Chloroform 400 ppm	Chloroform 900 ppm	Chloroform 1800 ppm
WBC × 10^3/mm^3	6.7	5.9[b]	6.6	6.4	5.0[b]	4.4[b]
RBC × 10^6/mm^3	7.84	7.98	8.17[b]	7.97	8.31[b]	7.97
Hgb, g-%	14.9	15.2	15.3[c]	15.2	15.6[b]	15.4[c]
Hct, %	43.1	44.6[c]	43.4	44.4	45.1[b]	44.4[c]
MCV, μ^3	55	56	53[b]	56	55	56
MCH, $\mu\mu g$	18.9	18.9	18.7	19.0	18.7	19.2
MCHC, %	34.4	33.9	35.3[c]	34.0	34.5	34.5
Differential						
PMN,%	20	19	16	14	17	16
Bands, %	0	0	0	0	0	0
Lymphocytes, %	78	79	81	84	80	81
Monocytes, %	2	1	1	1	2	1
Eosinophils, %	1	1	1	1	2	2
Basophils, %	0	0	0	0	0	0

[a]One sample clotted, therefore, only 19 samples were analyzed for this level. All other levels contained 20 samples. The differential count for this level contained 20 samples.
[b]$p \leq 0.01$.
[c]$p \leq 0.05$.

Table 6. Summary of hematology data for male Osborne-Mendel rats receiving chloroform in their drinking water: 18-month sacrifice.

Parameter	Control	Matched control[a]	Chloroform 200 ppm	Chloroform 400 ppm	Chloroform 900 ppm	Chloroform 1800 ppm
WBC × 10^3/mm^3	6.6	6.2	6.0	6.7	5.8	6.4
RBC × 10^6/mm^3	7.43	7.57	7.38	7.66	7.76	7.49
Hgb, g-%	14.4	14.7	14.4	15.0	14.9	14.4
Hct, %	41.6	42.8	41.4	42.8	42.5	41.5
MCV, μ^3	56	57	57	56	55	56
MCH, $\mu\mu g$	19.4	19.4	19.5	19.6	19.2	18.7
MCHC, %	34.8	34.3	34.7	35.2	35.0	34.7
Differential						
PMN,%	21	21	19	21	20	27
Bands, %	0	0	0	0	0	0
Lymphocytes, %	74	76	77	75	77	69
Monocytes, %	3	2	2	3	2	2
Eosinophils, %	2	2	2	1	1	2
Basophils, %	0	0	0	0	0	0

[a]One sample clotted; therefore, only 19 samples were analyzed for this level. All other levels contained 20 samples. The differential count for this level contained 20 samples.

potassium, phosphorus, bilirubin, alkaline phosphatase, total iron, albumin and the albumin/globulin ratio tended to be higher in treated groups than in the negative controls. Cholesterol, triglycerides, LDH and globulin tended to be lower in treated groups than in the negative controls. In the matched controls, glucose and total iron tended to be higher and cholesterol and triglycerides tended to be lower than in the negative controls. In summary, most of the changes in hematologic and blood chemistry parameters observed in the treated groups were also evident in the matched control group. Thus, these effects of chloroform appear to be secondary to reduced water and food consumption.

Histopathologic evaluations have been completed for 200 rats and 62 mice that were sacrificed or found dead during the first 20 months of the study. From the mortality data, it is apparent that these samples are highly biased both in time and toward negative control animals. The percentages of rats examined to date are 45, 12, 10, 5, 6 and 4% for the negative control, matched control, 200, 400, 900 and 1800 ppm groups, respectively. Corresponding percentages for the mice are 3, 4, 4, 6, 20 and 18%

Table 7. Summary of clinical chemistry data for male Osborne-Mendel rats receiving chloroform in their drinking water: 6-month sacrifice.

Parameter	Control	Matched control	Chloroform 200 ppm	Chloroform 400 ppm	Chloroform 900 ppm	Chloroform 1800 ppm
Glucose, mg-%	182	215[a]	180	187	201	193
BUN, mg-%	22	24.4[b]	21.6	20.6	21.2	26.3[b]
Creatinine, mg-%	0.6	0.6	0.6	0.6	0.7	0.7[a]
Uric acid, mg-%	2.0	2.9	2.1	2.1	2.5	2.9
Na^+, meq/l.	146	147	146	146	146	145
K^+, meq/l.	5.3	5.9	5.2	5.4	5.6	6.4[b]
CO_2, meq/l.	24.0	24.8	26.6	27.0	26.0	26.0
Cl^-, meq/l.	99.7	96.3	99.7	100	102[b]	102[b]
Calcium, mg-%	10.0	10.0	9.9	10.1	10.3[b]	10.5[b]
Phosphorus, mg-%	5.9	5.8	6.0	6.1	6.7[b]	7.4[b]
Blance, Na-(Cl + CO_2)	22	20	20	18.5[b]	18[b]	18[b]
Cholesterol, mg-%	111	89[a]	107	114	103	100
Triglycerides, mg-%	127	69[b]	120	116	68[b]	32[b]
Total bilirubin, mg-%	0.13	0.13	0.13	0.13	0.17[a]	0.19[b]
SGOT, mU/ml	180	170	135	127	114[a]	129
SGPT, mU/ml	114	101	81	82	47	91
LDH, mU/ml	1828	1169[b]	1195[b]	994[b]	821[b]	744[b]
Alkaline phosphatase, mU/ml	194	221	180[a]	173	165	197
Total iron, μg-%	182	193	170	188	209[b]	202
Total protein, g-%	5.7	5.9	5.6	5.6	5.7	5.7
Albumin (A), g-%	2.8	3.0[a]	2.8	2.8	3.0[b]	3.1[b]
Globulin (G), g-%	2.8	2.9	2.8	2.8	2.7[a]	2.6[b]
A/G	1.0	1.05	1.0	1.05	1.1[b]	1.2[b]

[a] $p \leq 0.05$.
[b] $p \leq 0.01$.

Table 8. Summary of clinical chemistry data for male Osborne-Mendel rats receiving chloroform in their drinking water: one-year sacrifice.

Parameter	Control	Matched control	Chloroform 200 ppm	Chloroform 400 ppm	Chloroform 900 ppm	Chloroform 1800 ppm
Glucose, mg-%	182	240[a]	183	220	220	221
BUN, mg-%	22	23	20[b]	21	21	24[b]
Creatinine, mg-%	0.7	0.8	0.7	0.8	0.7	0.8
Uric acid, mg-%	2.3	3.1	2.0	3.5	3.5	3.1
Na^+, meq/l.	146	149[b]	146	147	146	146
K^+, meq/l.	5.1	6.3[a]	5.1	6.6[b]	6.6	7.0[a]
CO_2, meq/l.	27	25	27	25	25	24[a]
Cl^-, meq/l.	99	103[b]	101[b]	99	101[b]	103[b]
Calcium, mg-%	10.6	10.5	10.4	10.8	10.9	10.7
Phosphorus, mg-%	5.4	6.1[a]	5.2	6.3[b]	6.1[a]	6.7[b]
Balance, Na-(Cl + CO_2)	20	21	18[a]	22	21	19
Cholesterol, mg-%	159	112[b]	146	169	127[b]	105[b]
Triglycerides, mg-%	323	138[b]	257	249	91[b]	34[b]
Total bilirubin, mg-%	0.1	0.1	0.1	0.1	0.2[b]	0.2[b]
SGOT, mU/ml	204	211	136[a]	221	166	163
SGPT, mU/ml	112	117	100	156	145	105
LDH, mU/ml	1386	1380	1065[b]	1169	1059[a]	668[b]
Alkaline phosphatase, mU/ml	167	148[a]	161	140[b]	174	218[b]
Total iron, μg-%	164	193[b]	181[a]	183[a]	213[b]	231[b]
Total protein, g-%	5.8	5.9	5.9	5.9	6.0	6.0
Albumin (A), g-%	2.6	2.8	2.7	2.4	2.8[a]	3.0[a]
Globulin (G), g-%	3.2	3.1	3.2	3.4[b]	3.2[b]	3.0[b]
A/G	0.8	0.9	0.9	0.7[a]	0.8	1.0[b]

[a] $p \leq 0.05$.
[b] $p \leq 0.01$.

Table 9. Summary of clinical chemistry data for male Osborne-Mendel rats receiving chloroform in their drinking water: 18-month sacrifice.

Parameter	Control	Matched control	Chloroform 200 ppm	Chloroform 400 ppm	Chloroform 900 ppm	Chloroform 1800 ppm
Glucose, mg-%	185	213	177	169	195	178
BUN, mg-%	32	24	36	26	22[a]	25
Creatinine, mg-%	0.8	0.7	0.9	0.7[a]	0.7[b]	0.7
Uric acid, mg-%	1.9	2.9[a]	2.)	2.0	2.2	2.2
Na^+, meq/l.	148	148	148	149	148	148
K^+, meq/l.	5.1	6.0	5.3	5.0	5.1	6.2[b]
CO_2, meq/l.	29	27	30	30	29	29
Cl^-, meq/l.	102	102	100[a]	101	102	104[a]
Calcium, mg-%	10.3	10.2	10.5	10.2	10.2	10.1
Phosphorus, mg-%	5.2	5.5	5.2	4.9	5.3	6.4[b]
Balance, Na-(Cl + CO_2)	17	19	18	18	17	15
Cholesterol, mg-%	233	157[b]	231	200	157[b]	121[b]
Triglycerides, mg-%	375	225	373	255	130[b]	41[b]
Total bilirubin, mg-%	0.1	0.1	0.1	0.1	0.1	0.1
SGOT, mU/ml	99	153[b]	106	99	122	92
SGPT, mU/ml	61	98[b]	70	68	82	82[a]
LDH, mU/ml	944	1005	1028	854	778	560
Alkaline phosphatase, mU/ml	122	170[b]	119	124	135	167[b]
Total iron, μg%	119	165[b]	147[a]	140[a]	161[b]	162[b]
Total protein, g-%	5.4	5.5	5.5	5.4	5.5	5.4
Albumin (A), g-%	2.2	2.3	2.2	2.2	2.5[b]	2.6[b]
Globulin (G), g-%	3.8	3.1	3.3	3.2	3.0[a]	2.8[b]
A/G	0.7	0.7	0.7	0.7	0.8[b]	0.9[b]

[a] $p \leq 0.05$.
[b] $p \leq 0.01$.

(including early deaths). Thus, assessment of the effect of chloroform on tumorigenesis is not possible at this time.

For the rats, the conditions listed in Table 10 have been observed in appreciable numbers in the indicated sites.

In addition, sporadic tumor diagnoses have been made in the rats in various organs, including disseminated lymphosarcoma; adenocarcoma in the GI tract; squamous cell carcinoma, undifferentiated sarcoma, and sebaceous gland adenoma in the ear; Schwannoma in the eye; fibrosarcoma in the heart; tubular carcinoma, clear cell adenoma, adenocarcinoma, transitional cell carcinoma, and hemangiosarcoma in the kidney; bronchiolar adenoma in the lung; osteosarcoma; fibroma, sarcoma, fibrosarcoma, fibroadenoma, and hemangiosarcoma in various body regions; squamous cell carcinoma in the nostril; carcinoma in the nasal cavity; parathyroid adenoma; basophilic carcinoma in the pituitary; adenocarcinoma in the prostate; squamous cell carcinoma and papilloma in the stomach; interstitial cell tumor in the testes; and carcinoma, cystadenoma, and adenoma in the thyroid.

There have been insufficient mice examined to identify any lesions of appreciable incidence. Sporadic tumors in the mice have been diagnosed as disseminated lymphoma, both histiocytic and lymphocytic; pheochromocytoma; alveolar/bronchiolar adenoma and carcinoma; mammary adenoma and adenocarcinoma; carcinoma and teratoma in the ovary and leiomyosarcoma in the uterine cervix.

Table 10. Conditions observed.

Site	Conditions
Adrenal cortex	Adenoma, hyperplasia, hypertrophy, vacuolation
Adrenal medulla	Pheochromocytoma, hyperplasia
Kidney	Cyst, calculi, glomerulonephritis, hydronephrosis
Lung	Arterial hypertrophy, atelectasis, congestion, interstitial pneumonia
Lymph nodes	Hemorrhage
Parathyroids	Hyperplasia
Spleen	Hematopoiesis, hemosiderosis
Stomach	Mineralization

Discussion

With administration of chloroform at constant levels in the drinking water, the daily doses received by the animals are a function of fluid consumption and body weight. Using the water consumption and body weights for the first and twenty-third months

for illustration, the calculated mean daily doses for the rats receiving 200, 400, 900 or 1800 ppm of chloroform were 34, 66, 143 and 305 mg/kg and 34, 69, 132 and 238 mg/kg, during months 1 and 23, respectively. For the mice, the corresponding values were 54, 95, 207 and 382 mg/kg and 31, 63, 150 and 309 mg/kg. Thus, the daily doses in the present study were comparable to those used in two previous studies (*2,3*) at the higher levels and extended below them at the lower levels.

If it is assumed that the water consumption and body weight data provide indices of food consumption, the increased survival and decreased body weight in rats receiving chloroform are consistent with the well-known increase in longevity associated with caloric restriction (*4*). Since caloric restriction also results in reduced age-specific incidences of most pathologic conditions associated with aging (*4*), it will be intresting to consider the pathologic findings from the present study when they become available. It is also evident that inclusion of matched controls in long-term studies is extremely important.

In the mice the effect of chloroform on survival was markedly different than that in the rats. It appears that some of the mice rejected the chloroform solutions during the first weeks to such a degree that they were unable to survive. In fact, the only behavioral effect of the chloroform that was discernible during this study was the unthrifty appearance (e.g., lack of vigor, lassitude) of the mice during this time. From the water consumption and body weight data, it is clear that caloric restriction in the mice surviving the first weeks occurred to a much lesser extent than in the rats, if at all.

The increase in liver fat content for the mice was clearly greater than for the rats. It is probable that, in addition to differences between the mouse and the rat in the metabolism of chloroform (*5, 6*), the greater caloric restriction in the rats may have been a factor in reducing the relative accumulation of fat in the liver.

While the trends observed in some of the blood chemistry parameters associated with chloroform administration in the rat may be related to a nephrotoxic effect of the chloroform, nephropathy is one of the findings in the negative controls, and no definitive statements about the nephrotoxic effects of chloroform in the present study can be made at this time.

Discussion of the gross necropsy and histologic information will have be delayed until the terminal sacrifices and the histopathologic evaluations have been completed.

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